Faculty of Resource Science and Technology

Potential use of *Lactococcus lactis* subsp. *lactis* IO-1 in Fermented Cococnut Juice

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgments have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in the candidature of any other degree.

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ABSTRACT

Due to its elevated dietary advantage for health and pleasant taste as well as standard use in bio-pharmaceutical and beverage production, coconut beverage (CB) has drawn significant attention over the previous decade. CB is regarded as an appealing innovative probiotic fruit drink among non-dairy probiotic production, which helps to prevent and alleviate many health problems. The primary objective of this research was to justify *Lactococcus lactis*-IO1's growth, survival and production performance in various forms of CB (unpasteurized CB (UPW) and pasteurized CB at 90 °C (PCW90), 80 °C (PCW80) and 70 °C (PCW70) for 5, 15 and 25 minutes. The four distinct forms of CB (UPW, PCW90, PCW80, and PCW70) were fermented with *L. lactis* IO-1 for 48 h at 30 °C and the total viability of the cells, total soluble solids (TSS), pH, total acidity (TA), reduction sugar (RS), polyphenol, antioxidant (AO) and toxicity were studied at 24 and 48 h. This study proved that the production of different forms of CB with probiotic *L. lactis* IO-1 enhanced cell viability, TA, total phenolic content (TPC) and AO. The viable cell (9.5 log CFU/mL), TA (0.98%) and TPC (65.79 μg/mL gallic acid equivalent) in the fermented UPW were significantly ($p<0.05$) greater at 48 h compared to PCW90, PCW80 and PCW70 observed. Significantly fermented UPW ($p<0.05$) displayed the highest 1, 2-diphenyl-2-picrylhydrazl (DPPH), total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP) radical-scavenging abilities at 48 h (63.03, 68.88 and 67.62%, respectively), compared to the three other culture broths. The levels of RS, TSS and pH were significantly ($p<0.05$) lower in the fermented UPW at 48 h than the other observed three media. In addition, the four culture broths have low larvicidal potential against the brine shrimp, although the degree of toxicity between the fermented culture broths varied. After 28 days of storage process at 4 °C, growth of *L. lactis* in fermented UPW, PCW90, PCW80 and PCW70 reduced slightly and reaching a considerably elevated viability cell population of 9.13, 8.4, 8.49, 8.59 log CFU/mL, respectively. Throughout the 28 days of the storage process, the pH and TSS value remained relatively constant at 4 °C. The results obtained indicates that the UPW sample fermented with *L. lactis* IO-1 could be useful for development of functional beverage not only shelf life but also nutraceutical with health benefit, and able to overpower the natural microbiota of UPW, thus safer for consumptionat lower production costs.

Keywords: *L. lactis* subsp. *lactis* IO-1, coconut beverage, antioxidant, toxicity, probiotic.

Potensi Penggunaan* Lactoccus lactis *IO-1* subsp. lactis *dalam Jus Kelapayang Ditapai

ABSTRAK

Disebabkan kelebihan diet untuk kesihatan dan rasa yang menyenangkan serta penggunaan standard dalam pengeluaran bio-farmaseutikal dan penyediaan minuman, minuman kelapa (CB) telah mendapat perhatian semenjak sedekad lalu. CB dianggap sebagai minuman buah probiotik yang menarik dan inovatif di kalangan pengeluar probiotik bukan tenusu, yang dapat membantu mencegah dan mengurangkan masalah kesihatan. Objektif utama penyelidikan ini adalah untuk, mengenalpasti pertumbuhan, ketahanan dan prestasi penghasilan Lactococcus lactis *IO-1 dalam pelbagai bentuk CB (CB tidak dipasteurisasi (UPW) dan CB dipasteurisasi pada 90 ℃ (PCW90), 80 ℃ (PCW80) dan 70 ℃ (PCW70) selama 5, 15 dan 25 minit. Kempat-empat CB telah ditapai dengan* L. lactis *IO-1 selama 48 jam pada suhu 30℃ dan jumlah kebolehhidup, jumlah pepejal larut (TSS), pH, jumlah keasidan (TA), gula penurun (RS), polifenol, antioksidan (AO) dan ketoksikan telah diperhatikan setiap 24 dan 48 jam. Kajian ini membuktikan bahawa penghasilan pelbagai bentuk CB dengan probiotik* L. lactis *IO-1 meningkatkan daya hidup sel, TA, jumlah kandungan fenolik (TPC) dan AO. Diperhatikan bahawasel hidup (9.5 log CFU/mL), TA (0.98%) dan TPC (65.79 μg/mL setara asid galik) dalam UPW yang ditapai adalah ketara ($p<0.05$) lebih tinggi pada 48 jam berbanding dengan PCW 90, PCW 80 dan PCW 70. UPW ($p<0.05$) dengan signifikan menghasilkandifenil-2-pikrilhidrazil (DPPH) yang tertinggi, jumlah kapasiti antioksidan (TAC) dan kuasa antioksidan penurunan ferik (FRAP) keupayaan memerangkap radikal yang paling tingi pada 48 jam (63.03, 68.88 dan 67.62%, masing-masing) berbanding tiga kultur cecair* *yang lain. Tahap RS, TSS, pH, jumlah kandungan tanin dengan ketara ($p <0.05$) adalah lebih rendah dalam UPW yang ditapai pada 48 jam berbanding tiga media yang lain. Selain itu, keempat-empat kultur cecair mempunyai potensi larvisidal yang rendah terhadap nauplii udang air masin, walaupun tahap ketoksikan antara media cecair yang ditapai adalah berbeza. Selepas 28 hari proses penyimpanan pada 4℃, pertumbuhan* L. lactis *dalam UPW, PCW90, PCW80 dan PCW70 yang ditapai kurang sedikit atau kekal agak berterusan dan mencapai populasi sel hidup yang tinggi iaitu 9.13, 8.4, 8.49, 8.59 CFU/mL masing-masing. Sepanjang 28 hari proses penyimpanan, pada 4 ℃ nilai pH dan TSS kekal tetap. Keputusan yang diperoleh menunjukkan bahawa sampel UPW yang ditapai dengan* L. Lactis *IO-1 boleh digunakan untuk pembangunan minuman berfungsi bukan sahaja untuk jangka hayat tetapi juga nutraseutikal dengan faedah kesihatan, dan dapat mengatasi mikrobiota jadi semula UPW, dengan itu lebih baik untuk pengambilan dengan kos pengeluaran yang rendah.*

***Kata kunci*:** L. Lactis subsp. lactis *IO-1*, *minuman kelapa, antioksidan, toksisiti, probiotik.*

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LIST OF ABBREVIATIONS

AAS	2, 2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid)
AOA	Antioxidant activitiy
AP	Active phenolic
ATP	Adenosine triphosphate
B. *lactis*	*Bifidobacterium lactis*
BSLT	Brine shrimp lethality test
C. *nucifera*	*Cocos nucifera* L. (Coconut)
CB	Coconut beverage
CFU/mL	Colony forming unit per militer
CW	Coconut water
DF	Dilution factor
DHA-P	Dihydoxyacetone phosphate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNS	Dinitrosalicylic acid
DPPH	1, 1-diphenyl-2-picryl-hydrazyl
FAO	Food agriculture organization
FBP	Fructose-biphosphate aldolase
FRAP	Ferric-reducing anti-oxidant power
FS	Fermented sample
GAE	Gallic acid equivalent
GAP	3-phosphate glyceraldehyde

GI	Gasto intestinal
G3PDH	Glyceraldehyde-3-phosphate dehydrogenase
JCM	Japanese collection Microoragnism
LA	Lactic acid
LAB	Lactic acid bacteria
LAF	Lactic acid fermentation
LB	Lactate bacteria
LC_{50}	Lethal concentration
LDH	Lactate dehydrogenase
LF	Lactate fermentation
L. *lactis*	*Lactococcus lactis* subsp. *lactis*
L. *acidophilus*	*Lactobacillus acidophilus*
L. *casei*	*Lactobacillus casei*
MAYPAN	Malayan yellow dwarf × Panama tall
MCW	Mature green coconut water
MHA	Mueller Hinton Agar
MRS	De Man Rogosa Sharpe
PCW	Pasteurized coconut water
PK	Pyruvate Kinase
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPM	Revolution per minute
S. *cerevisiae*	*Saccharomyces cerevisiase*
S.D.	Standard deviation

TA	Total acidity
TAE	Tannin acid equivalent
TAC	Total antioxidant capacity
TSS	Total soluble solid
TTC	Total tannic acid
TPC	Total phenol acid
UPW	Unpasteurized coconut water
UV	Ultraviolet
°Brix	Total soluble solid

CHAPTER 1

INTRODUCTION

1.1 Background

Food crops play an essential role in the livelihood of the entire world's population as they are harvested for human consumption due to their significant nutritional values. Food crops are mainly divided into six categories such as; grains, legumes, seeds and nuts, vegetables, fruits as well as herbs and spices. Some of these food crops, for example, *Attalea cohune* (cohune palm), *Bactris gasipaes* (peach palm), *Areca catechu* (Betel-nut palm) and *Elaeis guineensis* J. (oil palm) are harvested and processed into various products such as margarine and cooking oil. These particular features make these crops as potential economic growth since it becomes a significant source of income for the larger populations of farmers (Jerard et al., 2008). *Cocos nucifera* L. (coconut) fruit is one of the food crops that is grown commercially in the Southeast Asian countries such as Vietnam, Malaysia, The Philippines, Thailand, and Indonesia due to the climatic region of the areas. *Cocos nucifera* L. tree species belongs to the family *Arecaceae* (Palmae), the genus of *Cocos* and the subfamily *Cocoideae,* which consists of approximately 2800 species of 190 genera. It is usually planted in the coastal area and it is commonly referred to as "tree of life" as its fruit, tree, and leaves serve multiple purposes.

Among the tropical fruits, coconut is considered as an attractive fruit for food and industrial exploitation as can be seen as the fourth largest agricultural plant in Malaysia after palm oil, rubber, and paddy in aspects of cultivation region. About 37% of the production of coconut is exported to other nations around the globe whereas the remaining 63% is for the local market (Sivapragasam et al., 2008; Gordon et al., 2017). *Cocos* *nucifera* L., in the shape of a nut, develops on trees of variable heights based on the coconut type. The fruit reaches the maturity stage at 9 months old and it weighs approximately 1-2 kg, a substantial reduction from 3-4 kg during its premature stage.

Coconut beverage is a popular healthy beverage among the countries with a hot and humid climate, especially, in Southeast Asia and the Carribean islands. Its liquid provides natural antioxidants as well as various mineral elements such as calcium, potassium, sodium, and magnesium that are essential in replenishing the human body's electrolytes during perspiration (Ismail et al., 2007; DebMandal & Mandal, 2011; Marikkar & Madurapperuma, 2012; Jain et al., 2019). On average, coconut water contains about 5-8% of total soluble solids (TSS), of which the majority are sugars (3-7%) whereas the rest of minor components are alcohols, enzymes, amino acids, vitamin and nitrogenous compounds (Mahayothee et al., 2016; Jain et al., 2019). The nutritional value of coconut water can be improved by mixing coconut water with other beverages such as orange and lemon drinks to produce isotonic drinks which can provide an additional source of vitamins and can reduce dehydration. Several studies have shown various health benefits of coconut water such as in the management of blood pressure, stroke and urinary tract infection, antibacterial, anti-inflammatory and antioxidant activities that may assist to neutralize the production of reactive oxygen species produced from long term exercise (Fisher-Wellman et al., 2009; Kalman et al., 2012; Boonumma et al., 2014). The production of coconut water is established approximately 1/4 of the year following the rapid growth of coconut tree and the fruit's liquid achieves full volume within nine months before its water content decreases as the coconut fruit matures (Bruce et al., 2008; Prades et al., 2012; Priya et al., 2014). In addition to coconut water, the fruit can be processed into other food products such as coconut candy, coconut oil, coconut flour, and coconut milk as well as non-food products such as fiber husks and local handicrafts made from the coconut's copra (Sangamithra et al., 2013).

Coconut fruits with bigger size and attractive green color yield higher price in local markets. However, the coconuts with physical deformities such as cracked fruits and spoiled coconut juice are often not wanted by consumers and thus, ended up as fruit wastes in larger quantities. These fruit wastes can be processed and converted further into value-added products that can maximize the profit among the farmers. For instance, the coconut water in larger quantities can be fermented into bioethanol that can be used as a source of biomass energy to generate electricity and biofuel for diesel-powered vehicles (Sangamithra et al., 2013).

1.2 Problem Statement

The production of *Cocos nucifera* L. fruit throughout the world is dominated by Southeast Asian countries such as Vietnam, The Philippines, Thailand, Indonesia, and Malaysia with the latter produced 555,120 tons of coconut fruit in 2012 (Othaman et al., 2012). However, the overproduction of coconut results in an excess of fruits and increase in wastage to the environment. Since cococnut water has a limited shelf life at ambient temperature (25-27 °C) and becomes overripe rapidly after harvesting, reducing its quality, potency and health benefits for consumers. Inside the fruit, the water is sterile but as soon as it taken out exposed to air it becomes vulnerable to oxidation furthermore of microbial contamination (Matsui et al., 2008).

In order to overcome this problem, certain preservation techniques have been reported by Tan et al. (2014) to extend the shelf life of coconut water as shown in Figure 1.1. These techniques include using the thermal treatment, ultra, and microfiltration as well as sterilization to eliminate harmful microorganisms. However; these methods can cause the loss of bioactive compounds and nutrients with its entire delicate flavor is almost eliminated from the treated coconut water (Prades et al., 2012; Tan et al., 2014; Cappelletti et al., 2015).

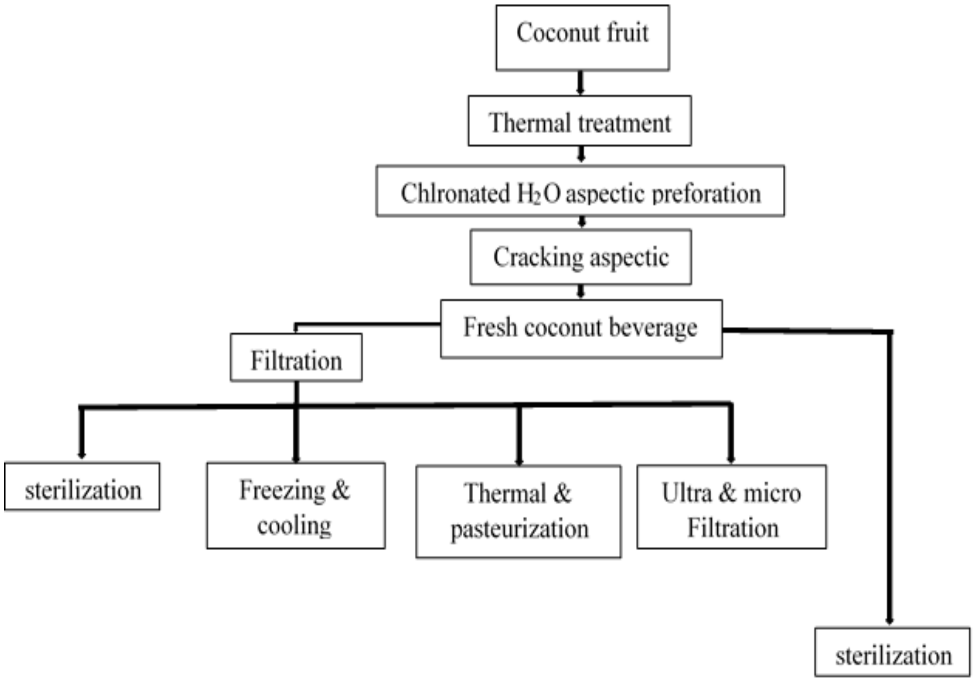

Figure 1.1: Conservation and processing of coconut water.

Fermentation serves as an alternative solution to enhance the preservation of the coconut water while making nutrients more accessible and improving its bioactive content. In this aspect, the fermentation process enables the shelf life to be extended and the organoleptic and physicochemical qualities of coconut beverage to be preserved through beneficial microorganisms. Coconut water kefir is a famous condiment in Indonesia and